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PRINCIPAL INVESTIGATOR: Wen-Hwa Lee, Ph.D.

CONTRACTING ORGANIZATION: University of Texas Health Science Center
at San Antonio, Center for Molecular Medicine
Institute of Biotechnology
San Antonio, Texas 78245

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Texas Health Science Center at San Antonio, Center for Molecular Medicine/Institute of Biotechnology
San Antonio, Texas 78245

E-MAIL:
leew@uthscsa.edu

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13. ABSTRACT ***(Maximum 200 Words)***

The objective of the program was to train highly qualified doctoral students in the genetic, cellular, and molecular basis of Breast Cancer. The training in Breast Cancer research which students obtain provided the scientific expertise for future discoveries in this important field. The training program, conducted within the Molecular Medicine Ph.D. Program, was administered by a select group of faculty whose research projects were intimately involved in breast cancer. An additional goal of the program was to promote synergistic interactions between the various laboratories engaged in breast cancer research. Breast cancer meetings, Molecular Medicine Distinguished Seminar Series and special Symposia were integral parts of the training program for students supported by the Breast Cancer Training Program. The major strengths of the program were the high quality of the Program faculty, and the interactive nature of the Breast Cancer research community in San Antonio. The program faculty was organized into four subprograms, which encompass scientists and physicians studying different aspects of breast cancer and cancer therapy, as well as fundamental mechanisms of cell growth and differentiation, DNA repair and molecular genetics. During the funding period, research by the students in the training program resulted in the publication of 40 peer-reviewed articles.

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10/18/99

PI - Signature Date

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INTRODUCTION

1. Brief Description of the Training Program and Its Objectives

The goal of the program was to establish at the University of Texas Health Science Center in San Antonio an in-depth training program in the Molecular Genetics of Breast Cancer. The most important goal of the program was to train highly qualified Ph.D. students in the genetic, cellular, and molecular basis of Breast Cancer. Toward these ends, the program has been extremely successful. Based on the publication record of our trainees, our expectation that the background in Breast Cancer Biology these students obtain will lead to significant future discoveries was realized. To date, a total of 40 publications relevant to breast cancer has been achieved by the students supported by the training program.

The training program was conducted within the Molecular Medicine Ph.D. Program by a select group of faculty whose research projects are relevant to breast cancer. An additional goal of the program was to promote synergistic interactions between the various laboratories engaged in breast cancer research. An important meeting was the Annual Breast Cancer Symposium held in San Antonio. All students supported by the program were required to attend. Finally, an outstanding Molecular Medicine Seminar Series sponsored by the Department of Molecular Medicine was also a requirement for all trainees. The following seminars in this series were pertinent to breast cancer:

- Fall Semester,1997:

G. Steven Martin	"Transformation by Src and Ras"
Winship Herr	"Transcriptional regulatory mechanisms"
Glenn D Preswich	"New affinity probes for cell signaling"
Robert Benezra	"Mitotic checkpoint controls"
Richard Baer	"The functional properties of BRCA1"
Alan M. Weiner	"A viral model for chromosome fragility"
Michael Lieber	"Site-specific recombination"
Larry H. Thompson	"Recombination repair in mammalian cells."

- Spring Semester, 1998:

Joanna Groden	"Genomic stability and inherited predisposition to cancer"
Kenneth Kraqemer	"Recent studies on DNA repair and Xeroderma Pigmentosum"
Eric R. Fearon	"Colorectal cancer genetics and the DCC gene"
Carlo M. Croce	"Genetics of human cancer"
John D. Minna	"Molecular pathogenesis of lung cancer"
David B. Roth	"DNA cleavage and joining in V(D)J recombination"

- Fall Semester, 1998:

Xiaodong Wang	"Biochemical studies of apoptosis: putting a colorful puzzle together"
Richard Kolodner	"Multiple mechanisms of mutation suppression"
Jerry Shay	"The regulation of telomerase in aging and cancer"
Lorraine Symington	"Mechanisms of DNA double-strand break repair"
Nicholas K. Tonks	Signal transduction and protein tyrosine dephosphorylation: from structure to function of protein tyrosine phophatases"
Charles M. Radding	"Molecular mechanisms of homologous recombination"
Joan Ruderman	"Cell cycle control"
Douglas Bishop	Assembly of meiotic recombination complexes"
Charles J. Sherr	"The ARF-p53 pathway in tumor surveillance"
Dan Finley	"Targeting proteins for breakdown by the proteasome"

M. Andrew Hoyt	"Mechanisms and regulation of mitosis in S. cerevisiae"
Satya Prakash	"The DNA repair, protein degradation, and chromatin silencing activities of yeast Rad6 ubiquitin-conjugating enzyme"
James N. Ihle	"Signaling by the cytokine receptor superfamily"

- Spring Semester: 1999

Hugo J. Bellen	"Genetic dissection of neurotransmitter release and endocytosis"
Riccardo Dalla-Favera	"Molecular genetics of B-cell lymphoma"
Cynthia McMurray	"Mechanism DNA expansion in human disease"
Stewart Shuman	"Mechanisms of DNA cleavage and rejoining"
Yue Xiong	"The regulation of p53 and pRb tumor suppression pathways"
Chien Ho	"From tracking cell movement to detecting organ rejection by MRI"
David S. Papermaster	"Retinal Degeneration induced in transgenic frogs"
John Petrini	"The Mre11/Rad50 protein complex mediates diverse function in the DNA damage response"
Michael Karin	"Protein kinase cascades that control AP-1 and NF-κB: regulation and function"
C.C. Wang	"Distinctive mechanisms regulating the proteasomes in a protozoan species, the Trypanosomes"
Francis Barany	"New methods of detecting genetic diseases and cancers"
Steven L. McKnight	New PAS domain proteins, new places, new biology"
Robert A. Sclafani	The role of Cdc7 and Cdk protein kinases in the cell cycle of yeast and human cancer cells"
Stephen A. Johnston	"Genetic immunization and other technologies that my revolutionize the ability to manipulate the immune response"
James C. Garrison	"The diversity of the G protein $\beta\gamma$ subunits and their role in cell signaling"
Richard W. Carthew	"The role of transcription repressors in photoreceptor cell development"

One of the major strengths of the program is the high quality of the Program faculty, and the interactive nature of the Breast Cancer research community in San Antonio. The program faculty was originally organized into four subprograms, which encompass scientists and physicians studying different aspects of breast cancer and cancer therapy, as well as fundamental mechanisms of cell growth, differentiation and molecular genetics. These faculty groupings are listed here, detailed descriptions of individual research programs were included in the original application.

A ***Breast Cancer Sub-Program***

C. Kent Osborne, M.D.
John Chirgwin, Ph.D.
Suzanne Fuqua, Ph.D.
E. Lee, Ph.D.
W. -H. Lee, Ph.D.
Z. Dave Sharp, Ph.D.
Patrick Sung, Ph.D.

B. Growth Factor Sub-Program

Gregory Mundy, M.D.
Robert J. Klebe, Ph.D.
Bettie Sue Masters, Ph.D.

D. Molecular Genetics Sub-Program

Robin Leach, Ph.D.
Peter O'Connell, Ph.D.
Alan E. Tomkinson, Ph.D.

In this final progress report, the relationship between the Breast Cancer Training Program and the Molecular Medicine Graduate Ph.D. Program is reviewed, and additional or updated information is provided regarding:

Research Support for Program Faculty
Listing of Supported Trainees
Project Summaries of upper level trainees
Appendix: Reprints of Trainee Publications

2. Relationship between the Breast Cancer Training Program and the Molecular Medicine Graduate Ph.D. Program

The Breast Cancer Training Program was implemented within the context of the Molecular Medicine Graduate Ph.D. Program. The Molecular Medicine Ph.D. Program is a recently established interdisciplinary Ph.D. training program in the Graduate School of Biomedical Sciences at the UTHSCSA. For the academic year 1998-9, there is a total of 53 students enrolled in the Molecular Medicine Program -- 45 Ph.D. and 8 M.S. Of those 53 students, only the Training Program in the Molecular Basis of Breast Cancer supported three.

The Breast Cancer Training program takes advantage of the internationally recognized breast cancer research programs existing in the institution for many years, and offers a unique opportunity for students interested in starting careers in breast cancer research. The participating scientists in this breast cancer program represent diverse departments including the Divisions of Medical Oncology, Hematology and Endocrinology in the Department of Medicine, and the Departments of Cellular and Structural Biology, Pathology and Biochemistry. In addition, the University of Texas Institute of Biotechnology and the San Antonio Cancer Institute [SACI], an NIH-designated Cancer Center, represent outstanding resources for training opportunities in clinical and basic science research. The national and international reputation of the participating faculty serve to attract a large number of excellent applicants to the breast cancer research track in the Molecular Medicine program. The continuation of a Breast Cancer Specialized Program of Research Excellent (SPORE) grant to the institution documents the quality of breast cancer research available to trainees.

The rationale for administering the breast cancer training program in the Molecular Medicine Ph.D. program was based on several important criteria: (1) The Molecular Medicine curriculum is specifically designed to provide basic science training while integrating fundamental principles of molecular biology with modern medicine. A Molecular Medicine Core course provides students with the mechanisms underlying human disease and provides intensive review of specific diseases [including breast cancer] that may serve as models for how human diseases can be studied at the molecular genetic level. (2) The Molecular Medicine program requires the participation of both clinical and basic scientists in the training process. The inclusion of MDs on all student advisory committees insures that every graduate has a clear perspective on the clinical relevance of the basic research in their program that in most instances will serve as a guide for the project. (3) The Molecular Medicine program is an interdepartmental, interdisciplinary program that offers flexibility to students in terms of research laboratories, advisors and committee members. This arrangement offers a real potential for synergism in breast cancer research not possible in traditional department-bound programs. In summary, the Ph.D. program in Molecular Medicine offers a near perfect environment for Ph.D. training in breast cancer and has attracted many well-qualified applicants.

3. Research Support for Program Faculty

An essential component of maintaining a successful and aggressive training program in Breast Cancer Research is the continued research funding of the individual Program Faculty laboratories. Current funding for each member of the Program faculty is detailed in Table 1. As can be readily seen from the table, the faculty has been extremely successful in obtaining research funding, including over $10,243,402 in direct costs for the 1998-1999 fiscal year.

4. Listing of Supported Trainees

Trainees who received support from the Training Program in the Molecular Basis of Breast Cancer Research are selected from among entering first year students in the Molecular Medicine Ph.D. Graduate Program. In subsequent years of their training, they may be maintained on the Training Program, or transferred to other funding sources, depending on the nature of their research interests, and the availability of grant support. The following trainees were supported on the Breast Cancer Training Program during the final reporting period.

Reporting Period 09/23/98 to 09/22/99

Shang Li – 4th year student
Stephen Van Komen – 2nd year
Qing Zhong – 3rd year

Record of Previous Year's Trainees:

Jim Fitzgerald	Graduated from the program with a M.S. degree.
Christa Hargraves	Left the program for academic reasons.
Zachary Mackey	Continues in the program as an upper level student [see report below].
Harold Pestana	Left the program for academic reasons.
Yuewei Qian	Graduated from the program with a Ph.D. Postdoc in James Maller's laboratory at the Howard Hughes Medical Institute at the University of Colorado School of Medicine. Dr. Qian's research involves understanding the cell cycle and cell proliferation. This is a problem that is relevant to all types of cancer, including those of the breast.

James Wang	Graduated from the Molecular Medicine Ph.D. Program, currently employed by GeneTex, Inc. Dr. Wang's work on the mechanism of viral latency is important in some cancers.
Linda deGraffenried	Continues in the Molecular Medicine Ph.D. Program as an upper level student [see report below].
Jennifer Gooch	Continues in the Molecular Medicine Ph.D. Program as an upper level student [see report below].
David Levin	Continues in the Molecular Medicine Ph.D. Program as an upper level student [see report below].
Ernesto Salcedo	Continues in the Molecular Medicine Ph.D. Program as an upper level student [see report below]. Ernesto was removed from the training grant since he elected to pursue work in a non-program faculty's laboratory [Dr. Steve Britt].
Jerry Alan Bates	Graduated from the Molecular Medicine Program with a M.S. from the laboratory of Dr. Robert Clark, Professor and Chair of the Department of Medicine whose work in on signal transduction.
Jill Gilroy	Continues in the Molecular Medicine Ph.D. Program as Ph.D. Student in the laboratory of Dr. Hanna Abboud, Professor and Chief of the Nephrology Division in Department of Medicine. Ms. Gilroy's work centers on signal transduction in kidney development.
Jonathan Mlocek	Resigned from the Molecular Medicine Ph.D. Program for personal reasons.
Ashby Morrison	Continues in the Molecular Medicine program as Ph.D. student in Dr. Kent Osborn's laboratory. She is working on identification and characterization of co-activators of the estrogen receptor and their role in the development of tamoxifen resistance during treatment of breast cancer.
Frank Yuan, M.D.	Graduated with at Ph.D. in 1999. Dr. Yuan has assumed a faculty position as an Assistant Professor in the Department of Obstetrics and Gynecology at the Kaohsiung Medical College in Kaohsiung, Taiwan.
John Leppard	Continues his training in the Molecular Medicine Program in the laboratory of Dr. Alan Tomkinson. See below for his contributions to the literature of DNA repair and DNA ligases, two subjects directly relevant to breast cancer.
Suh-Chin Lin	Continues her training in the laboratory of Dr. Eva Lee. Ms. Lin's research in the construction of animal models using conditional knock-out of p53 has successfully generated breast cancer-prone mice. These mice will be extremely valuable for future research applications directed toward development of novel therapeutic approaches and understanding breast tumor formation.
Hongyi Pan, MD	Dr. Pan graduated from the Molecular Medicine Program with a Masters degree in 1999. He has assumed a technical position in the Department of Pediatrics. See below for Dr. Pan's research that is being continued by another student, Dr. Zheng, and will soon be submitted for publication. This project identified a novel BRCA1-interacting protein (AP12), which has recently been shown to be a repressor of BRCA1-mediated transcription. Accordingly, this work is directly relevant to breast cancer.
Sean Post	Mr. Post continues his training in the laboratory of Dr. Eva Lee where he is working on understanding the function of the ATR gene product.

Lei Zheng, MD

Dr. Zheng continues his training in the laboratory of Dr. Wen-Hwa Lee where is working on the understanding the role of the retinoblastoma tumor suppressor protein in controlling the fidelity of chromosome segregation during cell division. Because RB is known to be implicated in breast tumor formation and/or progression, Dr. Zheng's work is highly relevant to breast cancer. Please see below for an additional discussion of Mr. Zheng's research.

The 1998-1999 academic year marks the sixth full year of operation for the Molecular Medicine Ph.D. Program, and was the final one for the Training Program in the Molecular Basis of Breast Cancer Research. The availability of highly qualified applicants to the Molecular Medicine Program was excellent. Overall, 87 applications were received for admission to the Fall 1999 entering class. Fifteen new students began classes in August of 1999. The total number of students at the start of the Fall semester 1998 in the Molecular Medicine Ph.D. Program at all levels was 53, which includes 18 women, and 5 minorities (1 African American and 4 Hispanic students). Three minority students have been supported by the Training Program in the Molecular Basis of Breast Cancer Research.

5. Project Summaries of Ph.D. Trainees

- ***Linda DeGraffenried*** ***Mentor -- Dr. Suzanne Fuqua***

Ms. deGraffenried's current project is to determine the cis-acting sequences responsible for the regulation of the human estrogen receptor gene. Deletion and site-directed mutagenesis of the ER promoter combined with transient transfection assays have revealed elements located proximal as well as distal to the primary transcriptional start site to be responsible. Mobility gel shift analysis suggests that a number of factors in whole cell extracts from ER-positive MCF-7 cells bind to the ER promoter between nucleotides -245 and -192, as indicated by the formation of four specific protein/DNA complexes. This region of the promoter contains a GC box between -223 bp and -211 bp as well as a non-consensus binding site for Sp1 between -203 bp and -192 bp. Antibodies to the transcription factors Sp1 and Sp3 supershift two of the specific complexes. Cotransfection of expression plasmids for Sp1 and Sp3 with an ER promoter-driven luciferase reporter plasmid into Sp1-void *Drosophila* SL2 cells induces a one-hundred- and a thirty-fold activation of the ER promoter, respectively. Transient transfection assays using linker-scanner mutants of the ER promoter spanning -245 bp to -182 bp also suggest an important role for elements flanking the Sp binding sites in the regulation of ER gene transcription. A detailed elucidation of these elements as well as the DNA-binding proteins that mediate transcriptional response will be characterized.

This project is directly relevant to breast cancer. Elucidating the basis for regulation of ER expression is an important issue in breast cancer research.

Publications:

Linda A. deGraffenried and Fuqua SAW. The Sp1 Transcription Factor Is Involved In Transcriptional Regulation of the Estrogen Receptor Gene in Human Breast Cancer Cells. Breast Cancer and Research Treatment 50: 333 (1998).

Linda A. deGraffenried, Welshons WV, Curran EM, and Fuqua SAW. Transcriptional Regulation of the Estrogen Receptor Gene Minimal Promoter in Human Cancer Cells. Endo '99 194 (1999).

Linda A. deGraffenried, Hilsenbeck SG, and Fuqua SAW. Sp1 is Essential for the Regulation of Estrogen Receptor Gene Transcription. J Biol Chem (submitted)

- ***David Levin*** ***Mentor -- Dr. Alan Tomkinson***

DNA joining events are required to maintain the integrity of the genome. Three human genes encoding DNA ligases have been identified. David is identifying the cellular functions involving the product of the LIG1 gene. Previous studies have implicated DNA ligase I in DNA replication and some pathways of DNA repair. During DNA replication, DNA ligase I presumably functions to join Okazaki fragments. However, under physiological salt conditions, DNA ligase I does not interact with DNA. It is Mr. Levin=B9s working hypothesis that DNA ligase I involvement in different DNA metabolic pathways is mediated by specific protein-protein interactions which serve to recruit DNA ligase I to the DNA substrate. To detect proteins that bind to DNA ligase I, David has fractionated a HeLa nuclear extract by DNA ligase I affinity chromatography. PCNA was specifically retained by the DNA ligase I matrix. To confirm that DNA ligase I and PCNA interact directly, Mr. Levin found that in vitro translated and purified recombinant PCNA bind to the DNA ligase I matrix. In similar experiments, he has shown that DNA ligase I interacts with a GST (glutathione S transferase)-PCNA fusion protein but not with GST. Using in vitro translated deleted versions of DNA ligase I, Mr. Levin determined that the amino terminal 120 residues of this polypeptide are required for the interaction with PCNA. During DNA replication PCNA acts as a homotrimer that encircles DNA and tethers the DNA polymerase to its template. He showed that DNA ligase I form a stable complex with PCNA that is topologically linked to a DNA duplex. Thus, it appears that PCNA can also tether DNA ligase I to its DNA substrate. A manuscript describing these studies has been published in the Proc. Natl. Acad. Sci. U.S.A.

In addition to interacting with PCNA, the amino terminal domain of DNA ligase I also mediates the localization of this enzyme to replication foci. To determine whether these are separable functions David fine mapped the region that interacts with PCNA and, in collaboration with Dr. Montecucco's group, the region required for recruitment to replication foci. Since the same 19 amino acids are necessary and sufficient for both functions and the same changes in amino acid sequence inactivate both functions, we conclude that DNA ligase I is recruited to replication foci by its interaction with PCNA. A manuscript describing these studies has been published in the EMBO Journal.

In recent studies, Mr. Levin has a constructed a mutant version of DNA ligase I that does not interact with PCNA. Importantly the amino acid substitutions do not affect the catalytic activity of DNA ligase I. By transfecting cDNAs encoding the mutant and wild type DNA ligase I into a DNA ligase I-mutant cell line, we will determine the biological significance of the DNA ligase I/PCNA interaction in DNA replication and DNA repair.

This project is relevant to breast cancer since problems with DNA replication and repair will undoubtedly be involved in the development of all tumors at some stage in their progression.

Publications:

Mackey, Z.B., W Ramos, **DS Levin**, CA Walter, JR McCarrey and AE Tomkinson. 1997 An alternative splicing event, which occurs in mouse pachytene spermatocytes, generates a form of DNA ligase III with distinct biochemical properties that may function in meiotic recombination. Molec. Cell. Biol. 17, 989-998.

Tomkinson, A.E. and **DS Levin** Mammalian DNA ligases. Bioessays. 18, 803-901 (1997)

David S. Levin W Bai, N Yao. and M O'Donnell and AE Tomkinson. 1997 Interaction between DNA ligase I and Proliferating Cell Nuclear Antigen; implications for Okazaki fragment DNA metabolism. Proc. Natl. Acad. Sci. U.S.A. 94, 12863-12868.

Montecucco, A., R Rossi, **DS Levin**, R Gary, MS Park, TA Motycka, G Ciarrocchi, A Villa, G Biamonti and AE Tomkinson. 1998 DNA ligase I is recruited to sites of DNA replication by an interaction with proliferating cell nuclear antigen: Identification of a common targeting mechanism for the assembly of replication factories. EMBO J. 17, 3786-3795

Matsumoto, Y., Gary, R., **Levin, D.S.**, Tomkinson, A.E. and Park, M. Reconstitution of long patch base excision repair with purified human proteins. *J. Biol. Chem* (1999) In Press

- ***Shang Li*** ***Mentor -- Dr. Wen-Hwa Lee***

Mutations of the *BRCA1* gene predispose women to the development of breast cancer. The *BRCA1* gene product [BRCA1] is a nuclear phosphoprotein whose cellular function is poorly understood. The C-terminal region of the BRCA1 protein contains an activation domain and two repeats termed BRCT (for BRCA1 C-terminal). In his recent work, Mr. Li identified a BRCT-interacting protein previously identified as CtIP, a protein that interacts with the C-terminal-binding protein (CtBP) of E1A. Together, CtIP and CtBP are postulated to form a transcription corepressor complex. The ability of BRCA1 to transactivate the p21 promoter can be inactivated by mutation of the C-terminal conserved BRCT domains. To explore the mechanisms of this BRCA1 function, the BRCT domains were used as bait in a yeast two-hybrid screen. A known protein, CtIP, a co-repressor with CtBP, was found. CtIP interacts specifically with the BRCT domains of BRCA1, both *in vitro* and *in vivo*, and tumor-derived mutations abolished these interactions. The association of BRCA1 with CtIP was also abrogated in cells treated with DNA-damaging agents including UV, γ-irradiation and adriamycin, a response correlated with BRCA1 phosphorylation. The transactivation of the p21 promoter by BRCA1 was diminished by expression of exogenous CtIP and CtBP. These results suggest that the binding of the BRCT domains of BRCA1 to CtIP/CtBP is critical in mediating transcriptional regulation of p21 in response to DNA damage.

This project is directly relevant to breast cancer since it involves the study of a protein whose function appears to central to the mobilizing the response of cells to DNA damage. Perturbations in the systems that maintain genomic integrity underlie initiation and progression of most cancers, including those of the breast.

Publications:

Chen C-F**, S. Li, Y. Chen**, P-L Chen, ZD Sharp, and W-H Lee. 1996 The Nuclear Localization Sequences of the *BRCA1* Protein Interact with the Importin-a Subunit of the Nuclear Transport Signal Receptor. **J. Biol. Chem.**, 271: 32863-32868 *Note: The three authors in bold contributed equally to this work.*

Liu, CY, A Flesken-Nikitin, **S. Li,** YY Zeng, and W-H. Lee. 1996. Inactivation of the mouse *Brca1* gene leads to failure in the morphogenesis of the egg cylinder in early postimplantation development. Genes Dev. **10**:1835-1843.

Shang Li, C-Y Ku, A. Farmer, Y-S Cong, C-F Chen, and W-H Lee. Identification of a novel cytoplasmic protein that specifically binds to nuclear localization signal motifs. J. Biol. Chem. 273:6138-6189 (1998).

Shang Li, Phang-Lang Chen, Thirugnana Subramanian, G. Chinnadurai, Gail Tomlinson, C. Kent Osborne, Z. Dave Sharp, and Wen-Hwa Lee. 1999 Dissociation of BRCA1 Binding to CtIP upon DNA Damage Mediates p21 Expression. J. Biol. Chem. 274:11334-11338

- ***Zachary Mackey*** **Mentor -- *Alan Tomkinson***

DNA joining events are required to maintain the integrity of the genome. Three human genes encoding DNA ligases have been identified. In this project we are intending to identify the cellular functions involving the product of the LIG3 gene. Mammalian cell lines with reduced DNA ligase III activity exhibit spontaneous genetic instability and increased sensitivity to DNA damaging agents. We have cloned human and mouse cDNAs encoding DNA ligase III. In both mouse and humans, we have identified two forms of DNA ligase III cDNA that differ at their 3' end and encode polypeptides with different C-termini. At the site where the cDNA sequences diverge, the nucleotide sequence resembles consensus splice donor/acceptor sequences. We have confirmed that these cDNAs represent alternatively spliced products from the same gene by cloning and analysis of the 3' end of the mouse LIG3 gene. Analysis of DNA ligase III expression by northern blotting demonstrated that this gene is highly expressed in the testes. Using RT-PCR, we have examined the expression of the two forms of DNA ligase III cDNA in mouse tissues and cells. One form of DNA ligase III mRNA, DNA ligase III-a is ubiquitously expressed. In contrast, expression of DNA ligase III-b mRNA is restricted to the testis. During spermatogensis, DNA ligase III-b mRNA expression occurs during the latter stages of meiotic prophase. This restricted expression pattern suggests that DNA ligase III-b mRNA may have a specific role in the completion of meiotic recombination. In support of this idea we have shown that DNA ligase III-a interacts with the DNA strand break repair protein Xrcc1 whereas DNA ligase III-b does not. We suggest that the DNA ligase III-a/Xrcc1 complex functions in DNA repair in both somatic and germ cells whereas DNA ligase III-b functions in meiotic recombination. A manuscript describing these studies has been published in Molecular and Cellular Biology.

A unique feature of the DNA ligases encoded by the LIG3 gene is an amino terminal zinc finger that binds to DNA single-strand breaks. This motif is not required for DNA joining vitro or for the functional complementation of an E. coli DNA =ligase mutant. However, the presence of this motif allows DNA ligase III to interact with and join nicked DNA molecules at physiological salt concentrations. Using site-directed mutagenesis, we have identified amino acid residues within the catalytic C-terminal domain that are required for interaction with nicked DNA. Our current working model is that the DNA ligase III zinc finger functions in vivo to displace another enzyme, poly (ADP-ribose) polymerase (PARP) from the nicks. A manuscript describing these studies has been submitted to the Journal of Biological Chemistry.

This project is relevant to breast cancer since genomic instability is likely to be involved at many of the several stages of breast cancer progression leading to malignancy. Methods to intervene and stabilize the genome could prevent progression and spread of the disease. In addition, information about DNA repair processes in normal and cancer cells may lead to the development of treatment regimes that more effectively kill cancer cells and minimize damage to normal tissues and cells.

Publications:

Wang, Y.-C.J., WA Burkhart, **ZB Mackey**, MB Moyer, W Ramos, I Husain, J Chen, JM Besterman and AE Tomkinson. 1994 Mammalian DNA ligase II is highly homologous with Vaccinia DNA ligase. Journal of Biological Chemistry 269, 31923-31928.

Husain, I., AE Tomkinson, WA Burkhart, MB Moyer, W Ramos, **ZB Mackey**, JM Besterman and J Chen. 1995 Purification and characterization of DNA ligase III from bovine testes. Journal of Biological Chemistry 270, 9683-9690.

Chen, J., AE Tomkinson, W Ramos, **ZB Mackey**, S Danehower, RA Schultz, JM Besterman and I Husain. 1995 Mammalian DNA ligase III: Molecular cloning, chromosomal localization and involvement in meiotic recombination during spermatogenesis. Molec. Cell. Biol. 15, 5412-5422.

Zachary B. Mackey, W Ramos, DS Levin, CA Walter, JR McCarrey and AE Tomkinson. 1997 An alternative splicing event, which occurs in mouse pachytene spermatocytes, generates a form of DNA ligase III with distinct biochemical properties that may function in meiotic recombination. Molec. Cell. Biol. 17, 989-998.

Tomkinson, AE and **ZB Mackey.** 1998 Structure and Function of Mammalian DNA ligases. Mutation Research. 407, 1-9.

Zachary B. Mackey, Niedergang, C., Menissier-de Murcia, J., Leppard, J., Au, K., Chen, J., de Murcia, G. and Tomkinson, A.E. DNA ligase III is recruited to DNA strand breaks by a zinc finger motif homologous to that of Poly (ADP-ribose) polymerase. *J. Biol. Chem.* 274, 21679-21687 (1999).

- ***Hongyi Pan*** ***Mentor -- Dr. Wen-Hwa Lee***

Mutations in the breast cancer susceptibility gene, *BRCA1*, is involved in the development of hereditary breast cancer. The *BRCA1* gene product [BRCA1] is a nuclear phosphoprotein whose function is not well understood. One of Mr. Pan's project is to identify BRCA1-interacting proteins. One protein identified in this screen, named AP12, is a zinc-finger-containing protein. Since AP12 has the hallmarks of a Krab-domain repressor protein, Mr. Pan first identified the recognition sequence necessary for DNA-binding. Next and in collaboration with Dr. Zheng, a fellow graduate student, he inserted this sequence into mammalian reporter constructs and demonstrated that AP-12 can, indeed, repress transcription. The hypothesis under test is that BRCA1 can influence negatively the expression of a repertoire of AP12-regulated genes. This control function may be important in BRCA1-mediated suppression of breast cancer.

This project is relevant to breast cancer since BRCA1 function is hypothesized to be involved in suppressing the formation of breast cancer.

- ***Qing Zhong*** ***Mentor -- Dr. Wen-Hwa Lee***

One of Mr. Zhong's project in Dr. Lee's laboratory is a study of the tumor suppressor protein, TSG101. *tsg101* was identified as a tumor susceptibility gene by homozygous function inactivation of allelic loci in mouse 3T3 fibroblasts. To confirm its relevance to breast cancer that was originally reported, antibodies specific for the putative gene product were prepared and used to identify cellular 46 kDa TSG101 protein. A full size 46 kDa TSG101 protein was detected in a panel of 10 breast cancer cell lines and 2 normal breast epithelial cell lines with the same antibodies. A full-length *TSG101* mRNA was also detected using rtPCR. These results indicate that homozygous intragenic deletion of *TSG101* is rare in breast cancer cells. In more recent work, Mr. Zhong demonstrated that TSG101 is a cytoplasmic protein that translocates to the nucleus during S phase of the cell cycle. Interestingly, TSG101 is distributed mainly around the chromosomes during M phase. Microinjection of antibodies selective for TSG101 during G1 or S results in cell cycle arrest and overexpression leads to cell death.

These data indicate that neoplastic transformation due to lack of TSG101 could be due to a bypass of cell cycle checkpoints.

Another more recent interest of Mr. Zhong is the role of the breast tumor suppressor BRCA1 in cancer formation. *BRCA1*, encodes a tumor suppressor that is mutated in familial breast and ovarian cancers. Mr. Zhong's work showed that BRCA1 interacts *in vitro* and *in vivo* with human Rad50, which forms a complex with hMre11 and p95/nibrin. BRCA1 was detected in discrete foci in the nucleus that colocalize with hRad50 after irradiation. Formation of irradiation-induced foci positive for BRCA1, hRad50, hMre11 or p95 were dramatically reduced in HCC1937 breast cancer cells carrying a homozygous mutation in *BRCA1*, but was restored by transfection of wild-type *BRCA1*. Ectopic expression of wild-type, but not mutated *BRCA1* in these cells rendered them less sensitive to the DNA damage agent, methyl methanesulfonate. These data suggest that BRCA1 is important for the cellular responses to DNA damage that are mediated by the hRad50-hMre11-p95 complex.

Mr. Zhong's work on TSG101 and, especially, BRCA1 are is highly relevant for breast cancer research. By understanding the interaction and functional role of BRCA1 in the DNA repair process could lead to a greater understanding of its role in tumorigenesis and to new forms of cancer therapy aimed at interactions with the repair proteins.

Publications:

Qing Zhong, CF Chen, Y Chen, PL Chen, and WH Lee 1997 Identification of Cellular TSG101 protein in multiple human breast cancer cell lines. Cancer Res. 57, 4225-4228.

Qing Zhong, Y Chen, D Jones, W-H Lee 1998 Perturbation of TSG101 protein affects cell cycle progression. Cancer Res. 58; 2699-2702.

Qing Zhong., Chen, C. F., Li, S., Chen, Y., Wang, C. C., Xiao, J., Chen, P. L., Sharp, Z. D., and Lee, W. H. (1999). Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response. Science 285, 747-750.

Ashby Morrison ***Mentor -- Dr. Kent Osborne***

Ms. Morrison worked in three labs, breast cancer research being the primary area of research in each lab. My first lab rotation, which was in the lab of Peter O'Connell, Ph.D., She was involved in the preliminary work of locating a gene that when mutated may be involved in process of metastasis. The second lab rotation was done in the lab of Jolene Windle, Ph.D. During the months I spent in this lab I was exposed to the technique of using mouse models to study breast cancer. Specifically, my project involved transgenic and knockout mice to research the effects of oncogenes and tumor suppressors on breast cancer development. During her third lab rotation, in the lab of Kent Osborne, MD., Ms. Morrison was involved in a more clinical area of breast cancer research. Her project was to study the effects of varying levels of estrogen receptor coactivators and corepressors during tamoxifen treatment. Ms. Morrison was accepted into Dr. Osborne's laboratory where she continues to make good progress on the identification of estrogen receptor-associated proteins that are hypothesized to be co-activator/repressor proteins.

Jennifer L. Gooch ***Mentor -- Dr. Doug Yee***

Dr. Yee's laboratory is interested in the growth regulation of breast cancer cells by insulin-like growth factors (IGFs). Data from several laboratories had suggested that interleukin-4 (IL-4) and IGFs share common signaling pathways. Since it was known that IL-4 could directly inhibit breast cancer cell proliferation, Jennifer began examining the potential overlap of growth stimulatory and growth inhibitory signaling pathways in breast cancer cells.

Ms. Gooch first confirmed that IL-4 was inhibitory for breast cancer cells. This inhibition was dependent on expression of the IL-4 receptor and blocking antibodies to the receptor neutralized the effects of IL-4. She discovered that IL-4's growth inhibitory effects were dependent on cell proliferation. Quiescent cells were not affected by IL-4. Moreover, IL-4 induced apoptosis in estradiol-stimulated cells. She documented apoptosis by morphologic change, TUNEL assay, PARP cleavage, DNA laddering and generation of a sub-G1 peak by flow cytometry. Thus, she has shown that IL-4 inhibits breast cancer cell growth by inducing apoptosis to some, but not all, growth stimuli.

Because IL-4 and IGF-I share a common signaling pathway through insulin receptor substrate protein-1 (IRS-1), it is possible that this molecule coordinates both growth promoting and cell death signals. It is also possible that additional signals generated by IL-4 are responsible for its growth inhibitory effects. To date, she has documented Stat-6 activation by IL-4. She has shown that IL-4 treatment induces Stat-6 binding to a synthetic oligonucleotide in gel mobility shift assays. She has also shown that IRS-1 is activated by IL-4 in responsive cell lines. However, IL-4 differs from IGF-I in its kinetics of IRS-1 activation. While IGF-I rapidly phosphorylates IRS-1 to high levels followed by rapid dephosphorylation, IL-4 causes tonic levels of IRS-1 to appear in the cell. Furthermore, it appears that IRS-1 is rapidly degraded after IGF-I treatment, while such degradation does not occur after IL-4. Preliminary evidence suggests that IRS-1 may be ubiquitinated after IGF-I treatment, but not IL-4. Her future projects involve the detailed characterization of these pathways and determination of their contribution to IL-4's growth inhibitory effects.

Finally, she has shown that interferon-gamma (IFNg) stimulates Jak/Stat activation in human breast cancer cells. As in other epithelial tumors, activation of Stat-1 and Stat-3 appear to be growth inhibitory compared to their function in lymphocytes.

This project is relevant to breast cancer since intracellular signaling pathways are almost certainly involved in the growth stimulation at some stage of mammary cell tumor development or progression. Since growth inhibitory (IL-4) and growth stimulatory (IGF-I) pathways may be coordinated through a single molecule, the precise definition of the mechanism of IL-4 action, as compared to IGF-I action, could define molecular targets to inhibit breast cancer cell growth.

Publications:

Jennifer L. Gooch, Lee AV, Yee D. Interleukin-4 (IL-4) induces growth inhibition and apoptosis in human breast cancer cells. *Cancer Research*. 58:4199-4205, 1998.

Yee D, Jackson JG, Weng C-N, **Gooch JL**, Lee AV. The IGF system in breast cancer. In: K. Takano, Hizuka N, Takahashi S-I (ed.). Molecular Mechanisms to regulate the activities of insulin-like growth factors, pp. 319-325: Elsevier Science B.V., Amsterdam, 1998.

Lee AV, Jackson JG, **Gooch JL,** Hilsenbeck SG, Coronado-Heinsohn E, Osborne CK, Yee D. Enhancement of the insulin-like growth factor signaling in human breast cancer: Estrogen regulation of insulin receptor substrate-1 (IRS-1) in vitro and in vivo. *Molecular Endocrinology,* 13(5): 787-796, 1999.

Jennifer L. Gooch, Van Den Berg CL, Yee D. Insulin-like growth factor (IGF) -I rescues breast cancer cells from chemotherapy-induced cell death: proliferative and anti-apoptotic effects. *Breast Cancer Research and Treatment,* In Press, 1999.

Jennifer L. Gooch, Yee D. Strain-specific differences in the formation of apoptotic DNA ladders in MCF-7 breast cancer cells. *Cancer Letters*, In Press, 1999.

Jennifer L. Gooch, Herrera R, Yee D. The role of p21 in IFN-gamma-mediated growth inhibition in human breast cancer cells. *Cell Growth and Differentiation*, Under Revision, 1999.

Lee AV, **Gooch JL**, Osterreich S, Guler B, Yee D. IGF-I-induced degradation of IRS-1 is mediated by the 26S proteosome and requires PI-3 kinase. *Molecular Cell Biology*, Under Revision, 1999.

Jennifer L. Gooch, Lee AV, Christy B, Yee D. The role of insulin receptor substrate-1 (IRS-1) in insulin-like growth factor -I (IGF-I)- and interleukin-4 (IL-4)-mediated growth effects in human breast cancer cells. *J Biol Chem*, Submitted, 1999.

Jennifer L. Gooch, Christy B, Yee D. STAT6 mediates growth inhibition and apoptosis in human breast cancer cells. In Preparation.

Jill Gilroy ***Mentor – Dr. Hanna Abboud***

Signal transduction pathways are a vital part of development, proliferation, and tumorigenesis. In my work, I am interested in the involvement of growth factors, primarily Platelet Derived Growth Factor (PDGF) and its receptor (PDGFR), in signaling pathways. PDGFRs are tyrosine kinase receptors and upon stimulation dimerize and autophosphorylate, which in turn induces many downstream signaling molecules including, Mitogen Activated Protein Kinase (MAPK), and Phosphatidylinositol 3-kinase (PI3K). One of my goals was to determine the role of PI3K and MAPK in mediating biological processes such as cell migration and proliferation by PDGFR activation. Activation of PI3K was assayed using thin layer chromatography of anti-phosphotyrosine immunoprecipitates. MAPK activation was measured by immune complex assay of MAPK immunoprecipitates and SDS-PAGE using anti-phospho-MAPK antibodies. Functional assays, chemotaxis and ^{3}H-thymidine assays, were also preformed to test for cell migration and proliferation respectively. Inhibitors of MAPK and PI3K were also used in these studies to further show the involvement of these pathways in the aforementioned biological processes.

This project is relevant to breast cancer since signal transduction pathways are a vital part of tumorigenesis.

Shyng-Shiou (Frank) Yuan, M.D. ***Mentor -- Dr. Eva Lee***

The response of mammalian cells to DNA damage is complex, involving cell cycle arrest, DNA repair and, under certain conditions, apoptosis. Cells from individuals with the recessive disorder ataxia telangiectasia (AT) are hypersensitive to ionizing radiation. ATM (mutated in AT) protein contains a PI-3 kinase domain and is predominantly localized in the nucleus. c-Abl, a non-receptor tyrosine kinase, interacts with ATM and is a substrate of ATM kinase. Dr. Yuan demonstrated that ATM, c-Abl, and Rad51, a homologue of bacterial RecA protein required for DNA recombination and repair, can be co-immunoprecipitated from cell extracts. c-Abl interacts with and phosphorylates Rad51 *in vitro*. This phosphorylation enhances complex formation between Rad51 and Rad52, which functions with Rad51 in recombination and repair. After g-irradiation, an increase in both tyrosine phosphorylation of Rad51 and association between Rad51 and Rad52 occurs in wild-type cells but not in ATM-/- or c-Abl-/- cells. These findings implicate the ATM/c-Abl signaling pathway in promoting the assembly of the recombinational repair machinery.

Nijmegen breakage syndrome (NBS) is a rare autosomal recessive disease characterized by microcephaly, immunodeficiency, chromosomal instability and high cancer risk. There are many common features shared by AT and NBS, including loss of cell cycle checkpoint and sensitivity to IR. It has been shown recently that the gene product of NBS, Nibrin, is a 95 kDa protein. We demonstrated that nibrin forms a stable complex with repair proteins Rad50 and Mre11. The Rad50/Mre11/nibrin complexes possess nuclease activities which are likely to be important for recombination, repair, and genomic stability.

Whether there is a biochemical link between ATM and p95 is being studied. This information will provide a biochemical basis for the A-T and NBS cellular phenotypes as well as the mechanism of IR sensitivity in these cells.

The recombinase, Rad51, plays a key role in homologous recombination. Multiple Rad51-interacting proteins including Rad52, Rad54 and RPA are also required for homologous recombination. Several labs have reported that the protein product of breast cancer susceptibility gene, BRCA2, interacts with Rad51 directly through its BRC domains. In normal cells, a redistribution of Rad51 protein, manifested as formation of Rad51 nuclear foci, is seen upon ionizing radiation (IR). We show that in cells harboring BRCA2 mutation, there is little IR-induced Rad51 foci formation. In addition, introduction of GFP-BRC/BRCA2 fusion protein but not GFP compromised IR-induced Rad51 foci formation. This study suggests a specific dependence of IR-induced nuclear distribution on BRCA2.

These projects are highly relevant to breast cancer since recent studies indicate that the protein product of breast cancer susceptibility gene BRCA1 interacts with Rad50 (Dr. Wen-Hwa Lee, personal communication). Furthermore, it has been reported that ATM carriers may have a higher risk of breast cancer.

Publications

Trujillo, KM., **Yuan, S-S F.,** Lee, E. Y-H P., and Sung, P. Nuclease activities in a complex of human recombination and DNA repair factors Rad50, Mre 11, and p95. J. Biol. Chem. 273: 21447-21450.

Shyng-Shiou (Frank) Yuan, Cox, L. A., Dasika, G.K. and Lee, E.Y.-H.P. Cloning and functional studies of a novel gene aberrantly expressed in *Rb-/-* mouse embryos. Dev. Biol. 207:62-75 (1999).

Chen, G., **Yuan, S.-S. F.**, Liu, W., Xu, Y., Trujillo, K., Song, B.-W., Cong, F., Goff, S.P., Arlinghaus, R., Baltimore, D., Park, M.S., Sung, P. and Lee, E.Y.-H. P. Radiation-induced Assembly of Rad51 and Rad52 recombination complex requires ATM and c-Abl. J. Biol. Chem. 274:12748- 12752 (1999).

Shyng-Shiou (Frank) Yuan., Lee, S.-Y., Chen, G., Song, M., Tomlinson, G. E., and Lee, E.Y.-H.P. BRCA2 is Required for Ionizing Radiation-induced Assembly of Rad51 Complex *in Vivo.* Cancer Res. 59: 3547-3551 (1999).

Suh-Chin(Jackie) Lin ***Mentor -- Dr. Eva Lee***

The tumor suppressor gene, p53, is frequently mutated in human tumors, including breast carcinoma. P53 null mice develop multiple spontaneous tumors, predominantly lymphoma and sarcoma, within the first 6 months of age. To establish a mouse model of p53-mediated mammary tumor development, a bigenetic approach employing the cre-loxp system was initiated by Ms. Lin. Through gene-targeting in embryonic stem (ES) cells, mice carrying floxed p53 genes in which exons 5 and 6 are flanked by the loxp sequence were generated. A second mouse line carrying a cre transgene under the control of mouse mammary tumor virus

LTR (MMTV-cre) has also been generated. Floxed p53 mice were mated with MMTV-cre transgenic mice to produce mice with p53 inactivation in mammary tissue. Indeed, we observed p53 excision in the tissues of double transgenic mice. In addition, adenoviral vectors carrying cre recombinase are being used to inactivate p53. These approaches should provide a mouse mammary tumor model for studies of mammary tumor progression resulting from p53 mutation and for testing therapeutic interventions of mammary tumorigenesis. The resulting mice have demonstrated interesting patterns of tumor development including those of the mammary gland. These animals will be valuable models for testing new approaches to breast cancer treatment and understanding its etiology.

Upon DNA damage, p53 protein becomes phosphorylated and stabilized, leading to subsequent activation of cell cycle checkpoints. It has been shown that ATM is required for IR induced phosphorylation on Ser15 residue of p53. Based on the involvement of p53 in mammary tumorigenesis and on the higher risk of ATM carriers for breast cancer, we have carried out studies to address the cancer susceptibility of ATM heterozygous and ATM null mammary epithelial cells by transplanting mammary gland to wild-type sibling mice. Initial studies have indicated differential checkpoint and apoptotic responses in cells harboring ATM mutation. These studies will establish whether ATM plays important roles in mammary tumorigenesis.

Both of these projects are highly relevant to breast cancer, especially the Ms. Lin's animal models which hold promise in terms of new therapies for breast cancer and its metastases.

Publications:

Lin, S-C., Skapek, S. X. and **Lee, E. Y.-H. P.** Genes in the RB Pathway and their Knockout in Mice. Cancer Biology 271:279-289(1996).

Sean Post — ***Mentor -- Dr. Eva Lee***

Recent studies indicate that breast cancer susceptibility genes,BRCA1 and BRCA2, are involved in DNA repair. Cells harboring mutations in either gene are hypersensitive to ionizing radiation (IR). Extensive genetic evidence in yeast indicates that DNA double-stranded breaks are processed by Rad50/Mre11 nuclease complex. It has also been shown that in response to IR, Rad50 assembles into nuclear foci. In mammalian cells, such IR-induced Rad50 foci are not observed in cells established from Nijmegen breakage syndrome (NBS). We and others have shown that the protein product of gene mutated in NBS, Nibrin, forms a stable complex with Rad50/Mre11 and the complex possesses nuclear activity. We demonstrated that IR-induced Rad50 redistribution requires ATM kinase activity. Rad50 is phosphorylated upon IR. Our preliminary studies indicate that such IR-induced Rad50 foci formation and phosphorylation are defective in A-T cells. In addition, IR-induced Rad50 foci formation is aberrant in some sporadic cancers that express normal ATM, Rad50, Mre11, nibrin, BRCA1 and BRCA2 suggesting involvement of additional protein in this DNA damage response.

Mr. Post is a third year graduate student who is characterizing IR-induced Rad50 phosphorylation. How phosphorylation affects Rad50 function will be studied. In addition, cross-linking experiments will be carried out to investigate whether there is defective Rad50 protein complex formation in breast cancer cells. These studies will provide insights into the role of ATM kinase cascade in the assembly of double-stranded breakage repair protein. Furthermore, characterization of components in the repair protein complex may lead to the identification of additional players involved in breast carcinoma.

These projects are highly relevant to breast cancer since genomic instability is a hallmark of cancer and is thought to be a major contributor to the tumorigenic process. Mr. Post's research will contribute toward a greater understanding of the mechanisms responsible for maintaining genomic integrity which is undoubtedly involved in breast cancer development and progression.

Lei Zheng **Mentor -- *Dr. Wen-Hwa Lee***

In his work, Mr. Zheng explored the role of the retinoblastoma tumor suppressor (Rb) in the process of chromosome segregation. A yeast homologue (scHec1p) of the Rb-associated protein hHEC was shown to be essential for survival of yeast. The human HEC protein rescues the lethal phenotype of the null-mutation of sc*hec1* by complementing a critical role in modulation of chromosome segregation. A temperature-sensitive (ts) mutation of hs*HEC1* leads to a high frequency of errors in chromosome segregation. hsHec1p binds to Rb at an IxCxE motif specifically during M phase. In yeast carrying a ts allele of hs*hec1,* the expression of wild-type Rb reduced chromosome segregation errors by approximately 5-fold, suggesting that Rb enhances the fidelity of chromosome segregation. These results may also help explain why $Rb^{+/-}$ cells convert to $Rb^{-/-}$ at high frequency by loss of the wild-type *Rb* allele. How Rb and HEC facilitate chromosome segregation is the continuing pursuit of Mr. Zheng.

This is research that is highly relevant to breast cancer. Aneuploidy is the hallmark of cancer cells, including those of the mammary glands. Also, RB mutations are implicated in breast cancer. Accordingly, understanding this new role for the RB is relevant for a full understanding of breast cancer development and may provide new avenues for the development of novel therapies.

Publications:

Hao, W., Luo, Z., **Zheng, L.**, Prasad, K., and Lafer, E. M. (1999). AP180 and AP-2 interact directly in a complex that cooperatively assembles clathrin. J Biol Chem *274*, 22785-94.

Lei Zheng, Chen, Y., and Lee, W. H. (1999). Hec1p, an evolutionarily conserved coiled-coil protein, modulates chromosome segregation through interaction with SMC proteins. Mol Cell Biol *19*, 5417-5428.

Lei Zheng, Chen, Y., Riley, DJ, Chen, PL., and Lee, WH. (1999) Retinoblastoma protein enhances the fidelity of chromosome segregation mediated by a novel coiled-coil protein, HsHec1p (Submitted).

Stephen Van Komen **Mentor – *Dr. Patrick Sung***

Mutations in the tumor suppressor genes *BRCA1* and *BRCA2* greatly increase the risk of breast cancers. Recent studies have indicated that the BRCA1 and BRCA2 proteins modulate the enzymatic machinery which repairs DNA double-strand breaks by homologous recombination. Ongoing studies address the mechanism of the recombinational repair machinery by dissecting the functions of various recombination factors from yeast and human cells. The trainee, Stephen Van Komen, has made highly significant progress toward achieving the goal of dissecting the functions of the human recombination factor Rad51B and the yeast recombination factor Rad54. Specifically, Mr. Van Komen has raised polyclonal antisera against the human Rad51B protein and, using baculoviral protein expression vectors, has expressed the Rad51B protein in insect cells and determined the kinetics of induction of Rad51B. Mr. Van Komen has recently developed a procedure for purifying the Rad51B protein to about 50% purity. In the coming months, Mr. Van Komen will refine the purification procedure, obtain highly purified Rad51B, and carry out its functional analysis. In addition, Mr. Van Komen has been making great stride toward characterizing the yeast recombinational repair factor Rad54 and its mutant variants. The results from Mr. Van Komen's studies will be important for delineating the role of recombinational DNA repair in breast cancer suppression.

Mr. Van Komen's research is directly relevant to breast cancer since double strand breaks in DNA and their repair is an issue pertinent to breast cancer. Since the tumor suppressor, BRCA2, interacts with Rad51, it is critically important to understand the biochemistry of this important enzyme in DNA repair.

Publications:

Petukhova, G., S. **Van Komen, S.** Vergano, H. Klein, and P. Sung. (1999) Yeast Rad54 promotes Rad51-dependent homologous DNA pairing via ATP hydrolysis-driven change in DNA double helix conformation. J. Biol. Chem. In press.

Sung, P. Trujillo, K., and **S. Van Komen.** (2000) Recombination factors of Saccharomyces cerevisiae. Mutation Research. In press.

John Leppard ***Mentor -- Alan Tomkinson***

Three genes, *LIG1*, *LIG3*and *LIG4*, encoding DNA ligases have been identified in the mammalian genome. Unlike the *LIG1* and *LIG4* genes, there are no homologues of the *LIG3*gene in lower eukaryotes such as yeast. Biochemical and genetic studies suggest that DNA ligase III participates in base excision repair and the repair of DNA single-strand break. A feature of DNA ligase III that distinguishes it from other eukaryotic DNA ligases is a zinc finger. In preliminary studies we have shown that this zinc finger binds preferentially to nicks in duplex DNA and allows DNA ligase III to efficiently ligate nicks at physiological salt concentrations. One specific aim is to determine how the zinc finger of DNA ligase III binds to DNA single-strand breaks but does not hinder access of the catalytic domain of DNA ligase III to ligatable nicks. The second specific aim is to reconstitute the base excision and single-strand break repair pathways mediated by DNA ligase III and to elucidate the functional consequences of interactions between DNA ligase III and other DNA repair proteins such as Xrcc1, DNA polymerase beta and poly (ADP-ribose) polymerase that participate in these repair pathways.

Mr. Leppard's research is directly relevant to breast cancer since the ligase enzymes are vital to the concluding most of reactions where breaks are generated during the repair reactions. Genomic integrity is involved in breast cancer and Mr. Leppard's research will contribute toward a great understanding.

Publications:

Mackey, Z.B., Niedergang, C., Menissier-de Murcia, **J., Leppard**, J., Au, K., Chen, J., de Murcia, G. and Tomkinson, A.E. DNA ligase III is recruited to DNA strand breaks by a zinc finger motif homologous to that of Poly (ADP-ribose) polymerase. *J. Biol. Chem.* 274, 21679-21687 (1999).

<u>6. Changes to the Program Faculty:</u> None during the last funding period.

<u>7. Course Changes:</u> None during the last funding period.

8. Appendix: Table 1 – Current Funding for Program Faculty.

SUMMARY: The Breast Cancer Training Program made excellent progress toward attracting and retaining excellently qualified students in breast cancer research. The students received a high level of training in the modern research methods and theory. Students supported by the program achieved a total of 40 publications on breast cancer. Combined with the basic instruction they receive in the Molecular Medicine Ph.D. Program, they will graduate as highly skilled researchers who will competitive effectively for post doctoral positions in the premiere breast cancer laboratories in the world.

TABLE 1. CURRENT FUNDING FOR PROGRAM FACULTY

PARTICIPATING FACULTY MEMBER	FUNDING AGENCY	IDENTIFYING NUMBER AND TITLE	PROJECT PERIOD	CURRENT YEAR DIRECT COSTS
Lee, W.-H.				
Active Support	NIH/NEI	2 RO1 EY05758-15 (W.H. Lee) Molecular Basis of Retinoblastoma Formation	03/01/98-02/28/01	247,380
	NIH/NCI	2 RO1 CA58318-06 (W.H. Lee) Cancer Suppression by the Retino-blastoma Genes	07/01/99-04/30/03	157,026
	NIH/NCI	5 P50 CA58183-07 (Project 5) SPORE in Breast Cancer Project 5 - Tumor Suppressor Genes in Breast Cancer Development *(C. Kent Osborne - Program Director)*	08/01/95-07/31/00	143,924
	NIH	5 PO1 CA30195-18 (Project 5) Biomarkers of Breast Cancer Project 5 BRCA-1 Malfunction in Breast Cancer *(C. Kent Osborne - Program Director)*	08/01/97-07/31/02	172,048
	Department of Defense	DAMD17 99 1 9402 Training Program in the Molecular Basis of Breast Cancer Research *(W.H. Lee - Program Director)*	08/01/99-07/31/03	233,333

TABLE 1. CURRENT FUNDING FOR PROGRAM FACULTY

PARTICIPATING FACULTY MEMBER	FUNDING AGENCY	IDENTIFYING NUMBER AND TITLE	PROJECT PERIOD	CURRENT YEAR DIRECT COSTS
	NIH/NCI	2 P30 CA54174-08 (Coltman) NIH/SACI Cancer Core Grant *(W. H. Lee - Program Leader)*	08/01/98-07/31/02	15,750 *(Salary Support Only)*
Osborne, C. K.				
Active Support	NIH/NCI	5 P50 CA58183 SPORE in Breast Cancer	09/30/95-07/31/00	1,850,816
	NIH/NCI	5 PO1 CA30195-17 (Osborne) Markers of Breast Cancer Evolution and Progression	08/01/97-07/30/02	1,245,400
	Susan G. Komen Breast Cancer Foundation	(No Number Assigned) (Osborne) Mechanisms of Tamoxifen Resistance	10/01/96-09/30/99	35,000
	NIH/NCI	K 12 (Osborne) Physician Scientist Training Grant in Oncology	09/01/97-08/31/02	349,364
	Zeneca, Ltd.	(No Number Assigned) (Osborne) A Double-blind Randomized Multi-center Trial Comparing the Efficacy and Tolerability of 125 and 250 mg of Faslodez in Post-menopausal Women with Advanced Breast Cancer	11/01/96-04/31/99	67,000

TABLE 1. CURRENT FUNDING FOR PROGRAM FACULTY

PARTICIPATING FACULTY MEMBER	FUNDING AGENCY	IDENTIFYING NUMBER AND TITLE	PROJECT PERIOD	CURRENT YEAR DIRECT COSTS
Chirgwin, J. *Active Support*	Veterans Administration	VA (Chirgwin) Associate Research Career Scientist	04/01/94-09/30/99	41,351
	U.S. Army	Breast Cancer Idea Grant (Chirgwin) Role of Autocrine Motility Factor in Osteolytic Metastasis	04/01/98-03/31/01	63,391
	NIH	1 RO1 (D. Shah) Regulation of Renin in Preeclamptic Hypertension	04/01/99-03/31/00	53,940
	Veterans Administration	VA Research Service Merit Award Role of Bone-derived TGF-beta in Skeletal Metastases	10/01/99-09/30/04	159,200
	NIH	2 PO1 CA40035 (Mundy) Effects of Tumors on the Skeleton -- Project 2/Role of Bone-derived IGFs in Bone Metastasis of Human Breast Cancer	07/01/99-06/30/04	150,000

TABLE 1. CURRENT FUNDING FOR PROGRAM FACULTY

PARTICIPATING FACULTY MEMBER	FUNDING AGENCY	IDENTIFYING NUMBER AND TITLE	PROJECT PERIOD	CURRENT YEAR DIRECT COSTS
Fuqua, S.				
Active Support	NIH/NCI	5 P50 CA58183 (Osborne) SPORE in Breast Cancer -- Project 2 Heat Shock Proteins and Drug Resistance	09/30/95-07/31/00	143,482
	NIH	5 PO1 CA30195 (Osborne) Markers of Breast Cancer Evolution and Progression -- Project 2 Molecular Variants and Overexpression of ER in Clinical Breast Cancer Development	08/01/97-07/31/02	190,139
Klebe, R.				
Active Support	NIH	1 R41 DE1255 (Klebe) Tissue Engineering of Oral Tissues	02/15/98-01/31/00	31,500
	San Antonio Cancer Institute (SACI)	(No Number Assigned) (Klebe) Role of MMP-8 in Melanoma Invasiveness	06/01/99-05/31/00	15,000

TABLE 1. CURRENT FUNDING FOR PROGRAM FACULTY

PARTICIPATING FACULTY MEMBER	FUNDING AGENCY	IDENTIFYING NUMBER AND TITLE	PROJECT PERIOD	CURRENT YEAR DIRECT COSTS
Leach, R.				
Active Support	NIH/NIAMS	RO1 AR44919 (Leach) Isolation of Genes for Paget Disease and Osteosarcoma	01/01/98-12/31/01	134,266
	NIH/NIAMS	RO1 AR44603 (Roodman) Pathobiology of the Osteoclast in Paget's Disease	09/01/98-08/31/02	149,359
Lee, E. Y.-H. P.				
Active Support	NIH/NINDS	1 RO1 NS37381-01 (E. Lee) ATM Signaling and Neurodegeneration	05/01/98-02/28/01	157,050
	Texas Higher Education Coordinating Board	ATP 3659-034 (E. Lee) ATM Protein in DNA Repair and in Breast Cancer Predisposition	01/01/98-12/31/99	79,201
	Susan G. Komen Breast Cancer Foundation	9709 (E. Lee) Study of ATM Expression in Breast Cancer Cells and Elucidation of ATM Function in p53-mediated DNA Damage Check Point Regulation	10/01/97-09/30/00	35,000
	NIH/SACI Antigen & Antibody Core	2 P30 CA54174-08 (E. Lee) Antigen and Antibody Production Shared Resource	08/01/98-07/31/03	90,056

TABLE 1. CURRENT FUNDING FOR PROGRAM FACULTY

PARTICIPATING FACULTY MEMBER	FUNDING AGENCY	IDENTIFYING NUMBER AND TITLE	PROJECT PERIOD	CURRENT YEAR DIRECT COSTS
Masters, B.				
Active Support	NIH/NIGMS	GM31296 (Masters) Prostaglandin 19- and 20- Hydroxylation by Cytochrome P450	07/01/97-06/30/01	147,355
	NIH/NIGMS	GM52419 (Masters) Structural & Functional Modularity in Nitric Oxide Synthase	04/01/96-03/31/00	134,123
	The Robert A. Welch Foundation	AQ1192 (Masters) Structure-Function Relationships in the FAD- and FMN-Containing Enzymes, NADPH-Cytochrome P450 Reductase and Nitric Oxide Synthase	06/01/99-05/31/02	52,000
	NIH/NHLBI	HL30050 (Masters) Structural Determinants of FAD- and FMN-Requiring Enzymes	04/01/98-03/31/02	156,300
Mundy, G.				
Active Support	NIH	MO1-RR01346 General Clinical Research Center *(Gregory R. Mundy - Program Director)*	12/01/98-11/30/03	1,490,490

TABLE 1. CURRENT FUNDING FOR PROGRAM FACULTY

PARTICIPATING FACULTY MEMBER	FUNDING AGENCY	IDENTIFYING NUMBER AND TITLE	PROJECT PERIOD	CURRENT YEAR DIRECT COSTS
	NIH	32-AR07464 Training Program in Bone and Mineral Metabolism	07/01/93-04/30/00	70,472
	NIH	RO1-AR28149 Cytokines and Bone Cell Function	04/01/98-03/31/00	110,702
	NIH	2 PO1 CA40035 (Project 4) Program Project Grant - Effects of Tumors on the Skeleton *(Gregory R. Mundy - Program Director)*	07/17/99-04/30/04	139,702
	NIH	2 PO1 CA40035 (Administrative Core) Program Project Grant - Effects of Tumors on the Skeleton *(Gregory R. Mundy - Program Director)*	07/17/99-04/30/04	48,727
O'Connell, P.				
Active Support	NIH	RO1 DK47482 (O'Connell) NIDDM Suspectibility Genes in Mexican Americans	09/30/93-09/29/98	216,481
	NIH/NCI	2 P50 CA58183 (Osborne) SPORE in Breast Cancer -- Project 4 *Molecular Genetics of Pre-Malignant and Pre-Invasive Breast Disease*	09/30/95-07/31/00	160,857

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PARTICIPATING FACULTY MEMBER	FUNDING AGENCY	IDENTIFYING NUMBER AND TITLE	PROJECT PERIOD	CURRENT YEAR DIRECT COSTS
	NIH	2 RO1 DK42273 (O'Connell) Genetic Epidemiology of NIDDM in Mexican Americans	04/01/96-03/31/01	418,134
	NIH/NCI	2 PO1 CA55261 (O'Connell) Molecular and Genetic Epidemiology of Gliomas	01/01/96-12/31/01	81,433
	NIH/NCI	4 PO1 CA30195 (Osborne) Markers of Breast Cancer Evolution and Progression -- Project 4 *A New Metastasis Gene on Chromosome 14*	08/01/97-07/30/02	101,955
	NIH	RO1 A143279 CCR5 Regulation and Promoter Variants in HIV-1 Infection	07/01/98-06/30/03	178,837
Sharp, Z. D. *Active Support*	NIH	5 PO1 AG14674-02 Nutritional Probe of the Aging Process *(Arlan Richardson - Program Director)*	05/01/98-04/30/03	79,419

TABLE 1. CURRENT FUNDING FOR PROGRAM FACULTY

PARTICIPATING FACULTY MEMBER	FUNDING AGENCY	IDENTIFYING NUMBER AND TITLE	PROJECT PERIOD	CURRENT YEAR DIRECT COSTS
	American Institute for Cancer Research	(No Project Number) (Sharp) The Efficacy of Diet and Chemopreventatives on Cancer Progression in a Novel Mouse Model Mimicking Human Tumorigenesis	01/31/99-12/31/99	75,000
Sung, P. ***Active Support***	Department of Defense	DAMD17-98-8247 (Sung) Interactions among BRCA1, BRCA2, and Components of the Recombination Machinery	06/01/98-05/31/02	98,000
	NIH	RO1 ES07061-05 (Sung) DNA Repair Genes and Proteins of the *RAD52* Group	01/01/95-12/31/99	137,570
	NIH	RO1 GM57814-01 (Sung) Formation and Processing of Recombination Intermediates	05/01/99-03/31/03	140,010
Tomkinson, A. ***Active Support***	NIH	2 RO1 GM047251-06 (Tomkinson) Cellular Function of Eukaryotic DNA Ligases	05/01/98-04/30/02	117,294

TABLE 1. CURRENT FUNDING FOR PROGRAM FACULTY

PARTICIPATING FACULTY MEMBER	FUNDING AGENCY	IDENTIFYING NUMBER AND TITLE	PROJECT PERIOD	CURRENT YEAR DIRECT COSTS
	Council for Tobacco Research	#3786AR1 (Tomkinson) DNA Nucleotide Excision Repair in Eukaryotes	01/01/97-12/31/99	43,478
	Nathan Shock Center/Meadows Foundation	(No Project Number) (Tomkinson) Cellular Functions of the Werner Syndrome Gene Product	07/01/98-12/31/99	10,000
	San Antonio Cancer Institute	(No Project Number) (Tomkinson) Characterization of the Protein-protein Interactions that are Required for the Function of DNA Ligase I in DNA Replication and DNA Excision	07/01/98-12/31/99	14,985
	Howard Hughes Medical Institute/UTHSCSA	Enrich S/G 3/Tomkins (Rodriguez/ Tomkinson)	10/01/98-09/30/99	10,102